Supplementary Information

Oxidative stress enhances the expression of 2′,3′-cyclic phosphate-containing RNAs

Megumi Shigematsu and Yohei Kirino

Supplementary Figure Legends

Figure S1. Identification of cP-containing tiRNAs by cP-RNA-seq

(A) Production of tiRNAs. Upon various stress stimuli, ANG cleaves the anticodon-loops of tRNAs to generate tiRNAs. ANG-generated 5′-tiRNAs contain a cP and can be captured by cP-RNA-seq. Standard small RNA-seq can only sequence the RNAs which contain 5′-P and 3′-OH ends, such as 5′-tRFs. “aa” stands for amino acid.

(B) Schematic representation of cP-RNA-seq and standard RNA-seq methods. Cellular RNAs can have various terminal phosphate states. While standard small RNA-seq captures the RNAs with 5′-P and 3′-OH ends, cP-RNA-seq specifically captures cP-RNAs. “AD” stands for adapter.

Figure S2. cP-RNAs identified by cP-RNA-seq

(A) Proportion of identified cP-RNAs annotated to the indicated RNAs.

(B) Proportion of the 5′-terminal nucleotide of the 5′-tRNA^{HisGUG} halves.

(C) Expression profiles of the 3′-tiRNAs derived from respective cyto tRNA isoacceptors. The color key was obtained based on $\log_2$ reads per million (RPM) of the 3′-tiRNAs.

Figure S3. Confirmation of the expression of tiRNAs by northern blots

Total RNAs extracted from SA-treated HeLa or U2OS cells were subjected to northern blots for the indicated tiRNAs.

Figure S4. Isodecoder sequences of the four selected cyto tRNAs

Clustal Omega was used to obtain the sequence alignment of isodecoders. Numbers in parentheses indicate the number of genes encoding the isodecoder. The most major 5′-tiRNA sequence in each isodecoder is shown as black bold characters. The sequences that were not identified or identified only as minor species (less than 10 reads in any library) are shown in grey. A dagger denotes identical sequences of 5′-tiRNAs in each tRNA. The nucleotides that are not identical among the detected 5′-tiRNAs (shown as bold) are marked as red dots.

Figure S5. Alignment patterns of cP-RNAs for 28S rRNA

The positions of representative cP-RNAs, cPR-28S-np1s, cPR-28S-np1l, and cPR-28S-np3806, are indicated.

Figure S6. Alignment patterns of cP-RNAs for LPP and SMG1 mRNAs

The positions of representative cP-RNAs, cPR-LPP and cPR-SMG1, are indicated.

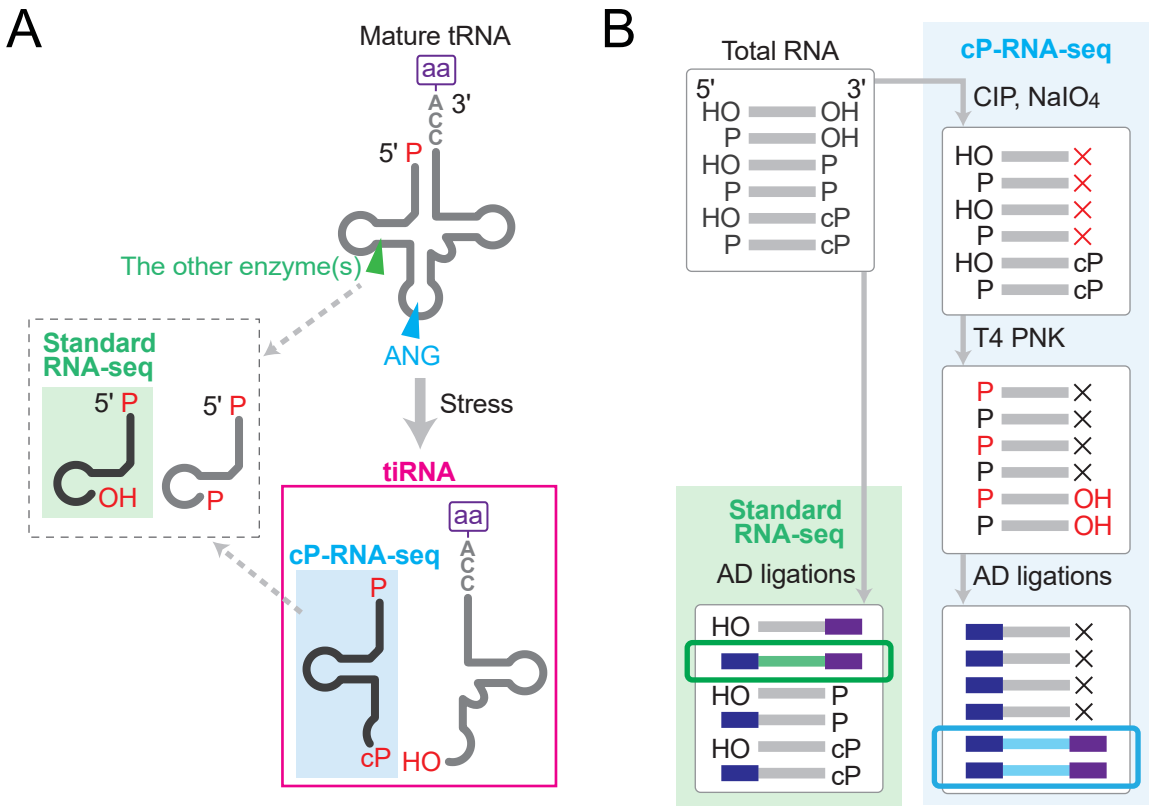
A
Mature tRNA
aa
ACC
3'
5' P
The other enzyme(s)
ANG
Stress
tiRNA
Standard
RNA-seq
5' P
OH
5' P
P
cP-RNA-seq
aa
ACC
P
cP
HO
B
Total RNA
5'
3'
HO
OH
P
OH
HO
P
P
P
HO
cP
P
cP
Standard
RNA-seq
AD ligations
HO
HO
P
P
HO
cP
cP
cP-RNA-seq
CIP, NaIO4
HO
P
HO
P
HO
cP
P
cP
T4 PNK
P
P
P
P
P
OH
P
OH
AD ligations

Figure S1

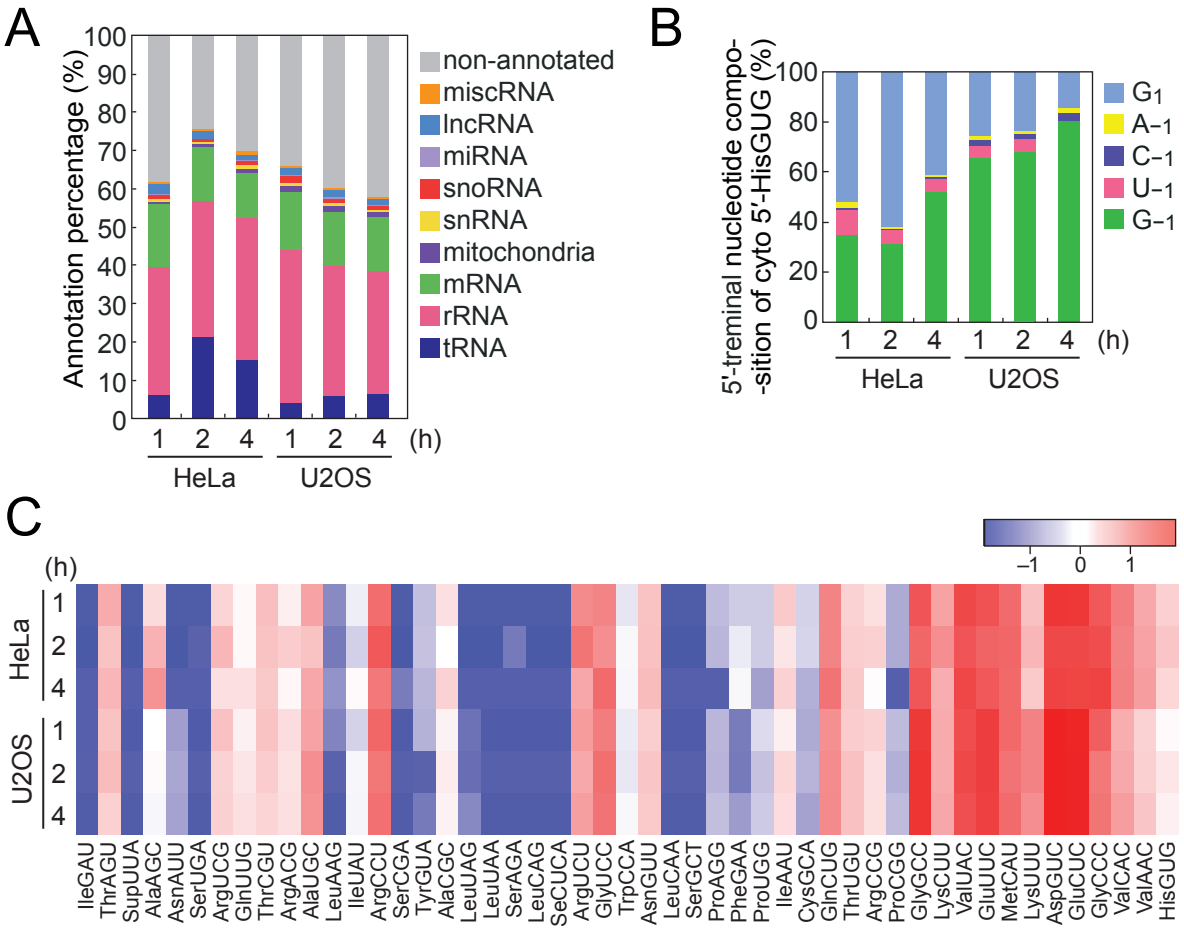

Figure S2

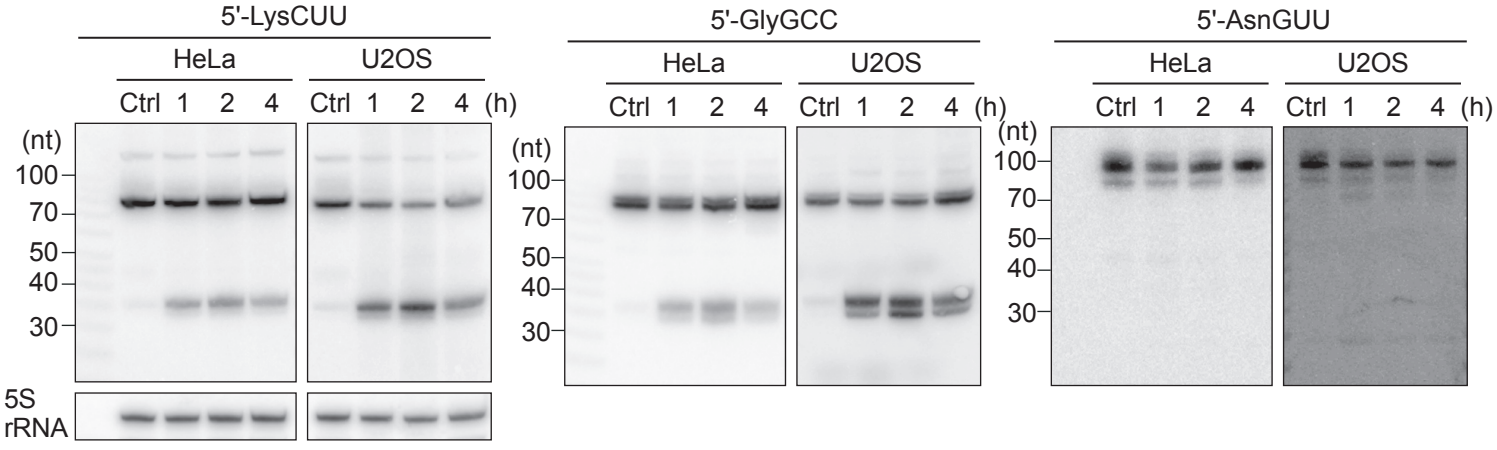

Figure S3

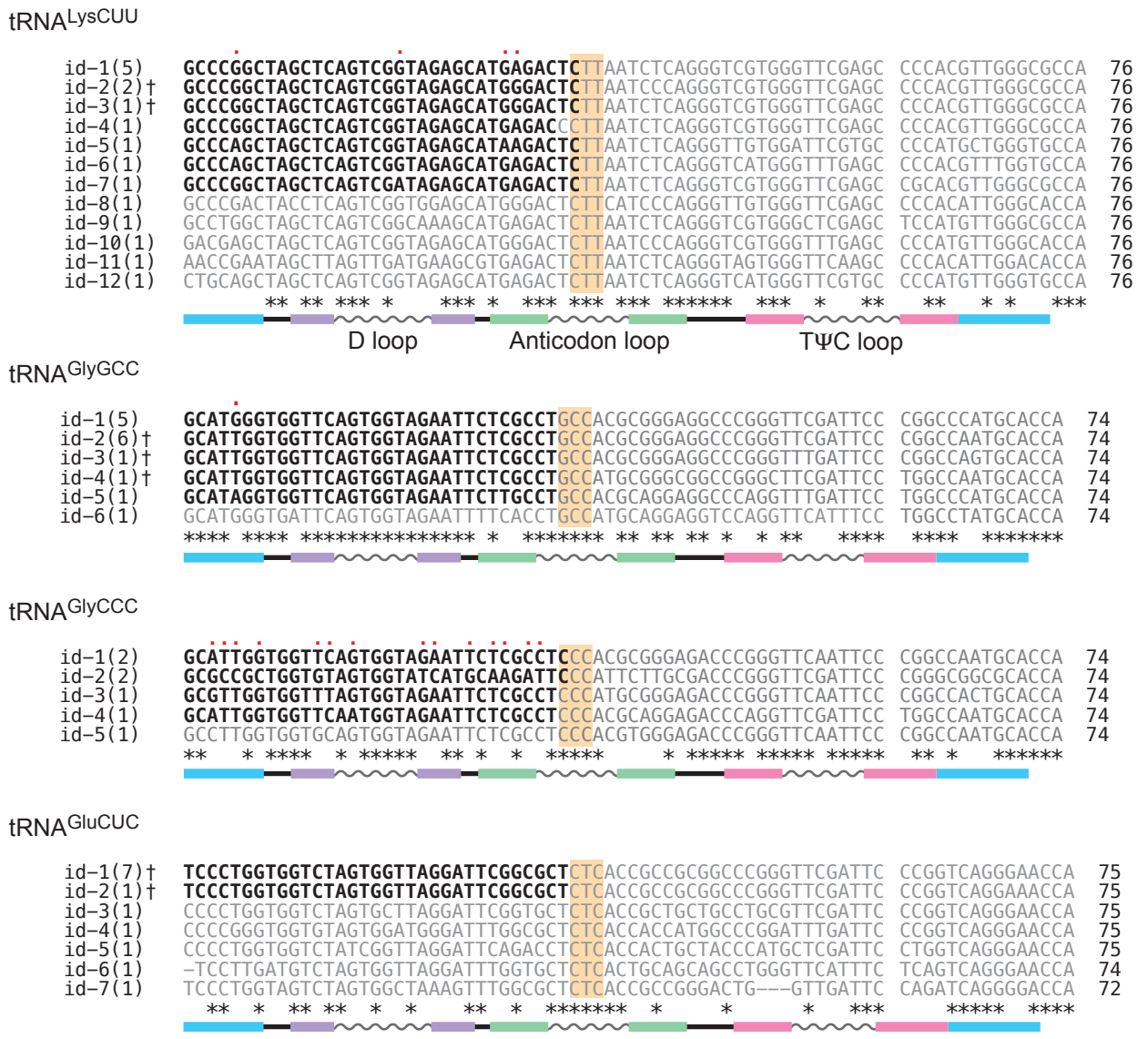
tRNALysCUU
id-1(5) GCCCGGCTAGCTCAGTCGGTAGAGCATGAGACTCTTAATCTCAGGGTCGTGGGTTCGAGC CCCACGTTGGGCGCCA 76
id-2(2)† GCCCGGCTAGCTCAGTCGGTAGAGCATGGGACTCTTAATCCCAGGGTCGTGGGTTCGAGC CCCACGTTGGGCGCCA 76
id-3(1)† GCCCGGCTAGCTCAGTCGGTAGAGCATGGGACTCTTAATCTCAGGGTCGTGGGTTCGAGC CCCACGTTGGGCGCCA 76
id-4(1) GCCCGGCTAGCTCAGTCGGTAGAGCATGAGACCCTTAATCTCAGGGTCGTGGGTTCGAGC CCCACGTTGGGCGCCA 76
id-5(1) GCCCAGCTAGCTCAGTCGGTAGAGCATAAGACTCTTAATCTCAGGGTTGTGGATTCGTGC CCCATGCTGGGTGCCA 76
id-6(1) GCCCAGCTAGCTCAGTCGGTAGAGCATGAGACTCTTAATCTCAGGGTCATGGGTTTGAGC CCCACGTTTGGTGCCA 76
id-7(1) GCCCGGCTAGCTCAGTCGATAGAGCATGAGACTCTTAATCTCAGGGTCGTGGGTTCGAGC CGCACGTTGGGCGCCA 76
id-8(1) GCCCGACTACCTCAGTCGGTGGAGCATGGGACTCTTCATCCCAGGGTTGTGGGTTCGAGC CCCACATTGGGCACCA 76
id-9(1) GCCTGGCTAGCTCAGTCGGCAAAGCATGAGACTCTTAATCTCAGGGTCGTGGGCTCGAGC TCCATGTTGGGCGCCA 76
id-10(1) GACGAGCTAGCTCAGTCGGTAGAGCATGGGACTCTTAATCCCAGGGTCGTGGGTTTGAGC CCCATGTTGGGCACCA 76
id-11(1) AACCGAATAGCTTAGTTGATGAAGCGTGAGACTCTTAATCTCAGGGTAGTGGGTTCAAGC CCCACATTGGACACCA 76
id-12(1) CTGCAGCTAGCTCAGTCGGTAGAGCATGAGACTCTTAATCTCAGGGTCATGGGTTCGTGC CCCATGTTGGGTGCCA 76
D loop
Anticodon loop
TΨC loop
tRNAGlyGCC
id-1(5) GCATGGGTGGTTCAGTGGTAGAATTCTCGCCTGCCACGCGGGAGGCCCGGGTTCGATTCC CGGCCCATGCACCA 74
id-2(6)† GCATTGGTGGTTCAGTGGTAGAATTCTCGCCTGCCACGCGGGAGGCCCGGGTTCGATTCC CGGCCAATGCACCA 74
id-3(1)† GCATTGGTGGTTCAGTGGTAGAATTCTCGCCTGCCACGCGGGAGGCCCGGGTTTGATTCC CGGCCAGTGCACCA 74
id-4(1)† GCATTGGTGGTTCAGTGGTAGAATTCTCGCCTGCCATGCGGGCGGCCGGGCTTCGATTCC TGGCCAATGCACCA 74
id-5(1) GCATAGGTGGTTCAGTGGTAGAATTCTTGCCTGCCACGCAGGAGGCCCAGGTTTGATTCC TGGCCCATGCACCA 74
id-6(1) GCATGGGTGATTCAGTGGTAGAATTTTCACCTGCCATGCAGGAGGTCCAGGTTCATTTCC TGGCCTATGCACCA 74
tRNAGlyCCC
id-1(2) GCATTGGTGGTTCAGTGGTAGAATTCTCGCCTCCCACGCGGGAGACCCGGGTTCAATTCC CGGCCAATGCACCA 74
id-2(2) GCGCCGCTGGTGTAGTGGTATCATGCAAGATTCCCCATTCTTGCGACCCGGGTTCGATTCC CGGGCGGCGCACCA 74
id-3(1) GCGTTGGTGGTTTAGTGGTAGAATTCTCGCCTCCCATGCGGGAGACCCGGGTTCAATTCC CGGCCACTGCACCA 74
id-4(1) GCATTGGTGGTTCAATGGTAGAATTCTCGCCTCCCACGCAGGAGACCCAGGTTCGATTCC TGGCCAATGCACCA 74
id-5(1) GCCTTGGTGGTGCAGTGGTAGAATTCTCGCCTCCCACGTGGGAGACCCGGGTTCAATTCC CGGCCAATGCACCA 74
tRNAGluCUC
id-1(7)† TCCCTGGTGGTCTAGTGGTTAGGATTCGGCGCTCTCACCGCCGCGGCCCGGGTTCGATTC CCGGTCAGGGAACCA 75
id-2(1)† TCCCTGGTGGTCTAGTGGTTAGGATTCGGCGCTCTCACCGCCGCGGCCCGGGTTCGATTC CCGGTCAGGAAACCA 75
id-3(1) CCCCTGGTGGTCTAGTGCTTAGGATTCGGTGCTCTCACCGCTGCTGCCTGCGTTCGATTC CCGGTCAGGGAACCA 75
id-4(1) CCCCGGGTGGTGTAGTGGATGGGATTTGGCGCTCTCACCACCATGGCCCGGATTTGATTC CCGGTCAGGGAACCA 75
id-5(1) CCCCTGGTGGTCTATCGGTTAGGATTCAGACCTCTCACCACTGCTACCCATGCTCGATTC CTGGTCAGGGAACCA 75
id-6(1) -TCCTTGATGTCTAGTGGTTAGGATTTGGTGCTCTCACTGCAGCAGCCTGGGTTCATTTC TCAGTCAGGGAACCA 74
id-7(1) TCCCTGGTAGTCTAGTGGCTAAAGTTTGGCGCTCTCACCGCCGGGACTG---GTTGATTC CAGATCAGGGGACCA 72

Figure S4

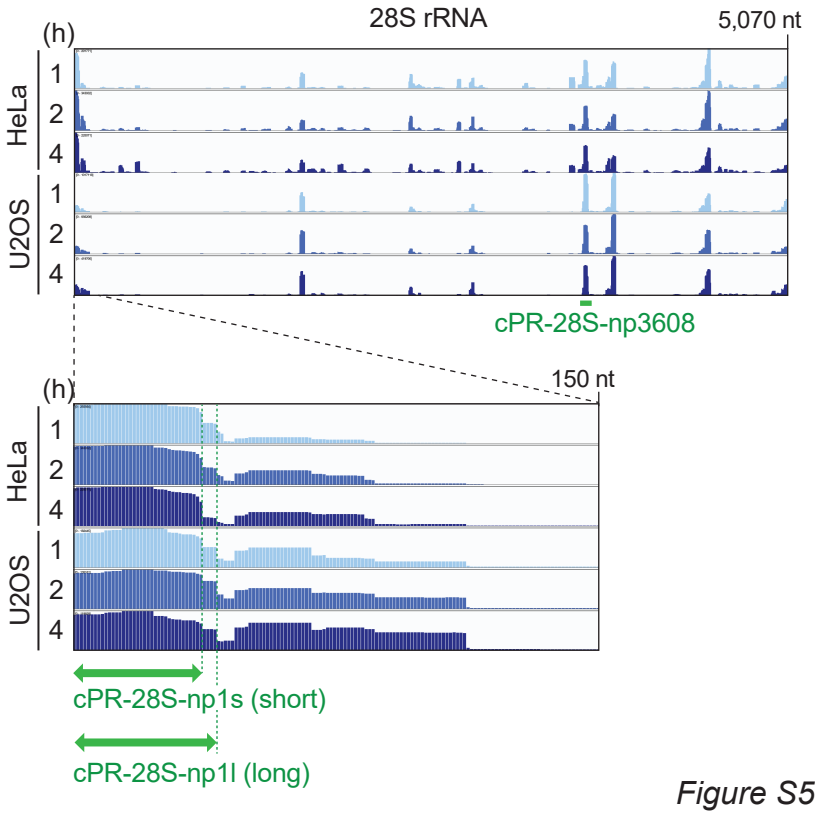

Figure S5

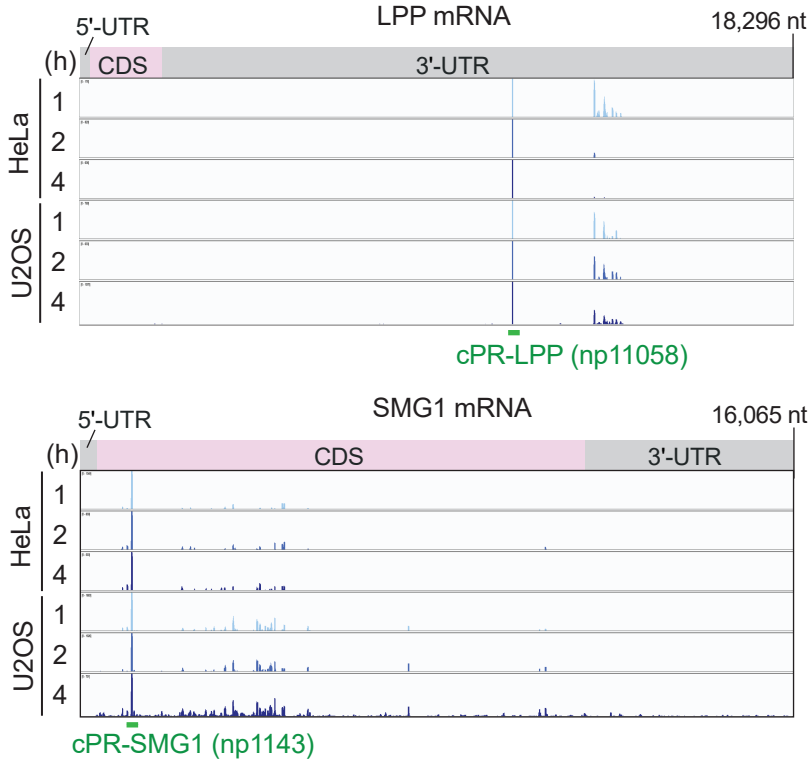

Figure S6

Supplementary Tables

Table S1. Read numbers of cP-RNA libraries obtained from cP-RNA-seq

		HeLa 1 h	HeLa 2 h	HeLa 4 h	U2OS 1 h	U2OS 2 h	U2OS 4 h
Total		49,594,334	64,937,613	45,795,016	116,510,986	86,468,731	73,853,430
Quality filter	:3′-AD trim	48,629,399	63,476,397	44,918,050	109,268,425	78,695,833	69,775,115
	:<20 nt	4,995,252	7,574,071	6,771,398	17,267,100	3,477,700	5,943,575
	:>45 nt	3,164,922	3,639,718	2,303,203	13,137,807	14,451,723	7,991,579
	:20–45 nt	41,434,160	53,723,824	36,720,415	86,106,079	68,539,308	59,918,276

Table S2. Sequences of TaqMan probes and primers for TaqMan RT-qPCR

Target	Probe/primer	Sequence (5′ to 3′)
cPR-28S-np1s	Forward primer	TACGGCCATACCACCCTGAAC
	TaqMan probe	/56-FAM/CGACCCGCT/ZEN/GAATTTGAACACTGCGT/3IABkFQ/
cPR-28S-np1l	Forward primer	TACGGCCATACCACCCTGAAC
	TaqMan probe	/56-FAM/CCCGCTGAA/ZEN/TTTAAGCGAACACTGCGTT/3IABkFQ/
cPR-28S-np3608	Forward primer	TCCGACTGTTTGAACACTGCG
	TaqMan probe	/56-FAM/TCCGACTGT/ZEN/TTGAACACTGCG/3IABkFQ/
cPR-LPP	Forward primer	AAATGGGCCGGGCGCGGT
	TaqMan probe	/56-FAM/CGTGCCTGT/ZEN/GAACACTGCGTT/3IABkFQ/
cPR-SMG1	Forward primer	AGGTCTCCAAGGTGAACAGCC
	TaqMan probe	/56-FAM/CATGTTGGA/ZEN/ACGAACACTGCGTTTG/3IABkFQ/
5S rRNA (control)	Forward primer	AAAGCACT AGAGACTGTAGGAG
	TaqMan probe	/56-FAM/CCTTTTCAT/ZEN/CTGTGAACACTGCG/3IABkFQ/

All synthetic probes and primers used in this study were synthesized by Integrated DNA Technologies (the abbreviations within the sequences are according to the company). For TaqMan RT-qPCR, universal reverse primer (5′-GATCGTCGGACTGTAGAACTC-3′) was used. TaqMan probes contain 6-carboxyfluorescein (FAM) as the fluorophore and ZEN/Iowa Black as the quencher.